Models in stress research

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published in
Ecological Complexity
2017

DOI (link to publisher)
10.1016/j.ecocom.2017.07.006

document version
Version created as part of publication process; publisher's layout; not normally made publicly available

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citation for published version (APA)
Kooijman, S. A. L. M. (2017). Models in stress research. *Ecological Complexity*, *34*, 161-177. https://doi.org/10.1016/j.ecocom.2017.07.006

Ecological Complexity xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Ecological Complexity

journal homepage: www.elsevier.com/locate/ecocom

Original Research Article

Models in stress research

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ARTICLE INFO

Article history:
Received 15 November 2016
Received in revised form 20 June 2017
Accepted 17 July 2017
Available online xxx

Keywords:
Empirical cycle
Dynamic systems
Model criteria
Statistics
Experimental design
Uptake and elimination routes
Compartment and film models
Chemical transformation
Dynamic energy budget theory

ABSTRACT

Mathematical models (should) play a central role in quantitative research, both in the design of experiments and in the analysis of their results. This also holds for research on stress on individual organisms, where stress is defined as an environmentally induced change in their (eco-physiological) behaviour, implying the necessity to know the behaviour in absence of stress in some detail. The individual can effectively be modelled in terms of a dynamical system, where stress shows up as a change in one or more parameters that control the behaviour of the system. After a more detailed presentation of the empirical cycle and an introduction to dynamic systems, I will discuss this approach in the context of generalised ecotoxicity, where presence (e.g. toxicants) or absence (e.g. dioxygen) of particular chemical compounds in the environment might affect a variety of endpoints (feeding, growth, reproduction, maintenance, survival). To this end I will discuss chemical transformation in the environment (speciation, ionisation, degradation, absorption), transport to and from the individual (various uptake and elimination routes, popular transport models), metabolic transformation, effects of nutritional status on kinetics and effects (lethal and sublethal).

1. Introduction

During my first exposure to ecotoxicity research in 1977 at the TNO laboratories in Delft, the Netherlands, I found myself between a group working on toxico-kinetics and one on effects, but they hardly interacted. With my background in theoretical biology, it was directly clear to me that molecules of any compound should first be in the neighbourhood of an individual before they could possibly have an effect. This coupling between toxico-kinetics and effects was the topic of my first paper in ecotoxicity (Kooijman, 1981). My next surprise during that first week of work in ecotoxicity was that people studied effects on the various endpoints (e.g. growth and reproduction) as if they are independent. I directly thought: if growth is reduced by eating less, how can it be that reproduction is not effected? There must be a coupling between effects on the various endpoints. To organise my thoughts on this took me a bit longer (Kooijman and Metz, 1984) and did send me deep down to the fundamentals of ecology and physiology, a life-filling enterprise that I later called the Dynamic Energy Budget (DEB) theory (Kooijman, 2010). It took long for DEB theory to became widely accepted. Apart of being more complex, involving the interaction of quite a few variables, I think that the main reason for this delay is the weak development of abstract thinking in biology. Many published models in biology suffer from dimension problems, illustrating the general lack of critical thinking about models. Even more frequently people seem to think that models are formulas that serve the task of describing data. I think, however, that a pencil does a better job than a model when it comes to describing data and that a formula itself is not really informative. The crucial information is in the assumptions behind a formula that generate it; different sets of assumptions can generate the same formula. I mentioned these two examples, linking toxicokinetics to effects and linking effects on different endpoints, to illustrate that some training in abstract thinking helps to see the broad picture. It affects the way you look at the world and the type of questions that jump into your mind.

A statement that is frequently heard from people with a distaste for models, is: ‘a model is not more than you put into it’. If done in the proper way, this is absolutely right and it is the single most important aspect of the use of models. Put into other words: any mathematical statement is either wrong or follows from assumptions. Few people throw mathematics away for this reason. Many biologists think that mathematics is difficult and have problems to understand how you go from one equality sign to another. Yet I think that mathematics is the only discipline that you really can understand (if you start at the beginning) and most frequently used math is actually very simple. While very good math books exist to help dealing with its technicalities, both elementary and advanced, the issue is in abstract (formalised) thinking behind the symbols which needs frequent practising and cannot start too early in ontogeny, like many other skills in life.

http://dx.doi.org/10.1016/j.ecocom.2017.07.006
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Knowing the chess rules do not make you a good chess player. Although training in abstract thinking is essential, it is not enough. The real challenge, as I see it, is not in mathematical derivations as such, but in stepping from the real world into the abstract one, and back again. These two crucial steps are not part of mathematics and require knowledge both on (parts of) the real as well as the abstract world. Linking abstract and real worlds is a discipline in itself. Happy enough, good books on this topic presently exist (van den Berg, 2011; Doucet and Sloep, 2011).

To make this point as explicit as possible, I first discuss the empirical cycle as I see it, and then the concept of dynamic systems as intro's to various aspects of understanding stress in individuals.

2. Empirical cycle

This paper discusses the empirical cycle in some detail, since experience learns that it is at the heart of a lot of misunderstandings, or at least disagreements, about the role of models in research and requirements that should be imposed on models to let them have this role: the empirical cycle is essentially about the interplay between the real and the abstract worlds to improve our understanding of the real world. Some empiricists do not seem to realise that measurements need interpretation before conclusions can be obtained from data, and, whether you like it or not, these interpretations involve models, even if not formalised. Given that the use of models in unavoidable, it is best to deal with them explicitly, to remain in control of the (otherwise implicit) assumptions. Few models in the literature are, however, derived from assumptions; they are simply posed, or even just coded. Such models are less suitable for application in the empirical cycle. The most important aspect of modeling, as I see it, is to make all assumptions explicit. If modeling procedures are followed in a sloppy way, by adapting models to fit data directly, it is likely that the conclusions from data will be sloppy too; one easily falls in the trap of curve-fitting in the sense of data description without helping understanding. If such a model fails one of the tests, nothing is left and one should start again from scratch. There cannot be a sequence of stepwise improvements in understanding and prediction. The fact that such a model fits data is of little use, perhaps only for interpolation purposes.

Models are idealizations and, therefore, always 'false' in the strict sense of the word. This limits the applicability of the principle of *falsification*. A model can fit data for the wrong reasons, which means that the principle of *verification* is even more limited in applicability. This points to the criterion usefulness to judge models, but *usefulness* is linked to a purpose. This is why a model should never be separated from its purpose. The purpose can contain elements such as increase in understanding, or in predictability. Increase in understanding can turn a useful model into a less useful one.

If a model passes all tests, including those against experimental data, there is no reason to change the assumptions, and work with them until new evidence forces reconsideration. It might seem counter intuitive, but models that fail the test against experimental data more directly serve their task in leading to greater insight, i.e. in guiding to the assumptions that require reconsideration. This obviously only works well if the steps of the formulation of assumptions have been adequate. Models are a mean in getting more insight, never an aim in themselves.

The next subsections highlight some steps in the two-segment empirical cycle, following the boxes in Fig. 1. Table 1 gives some practical hints.

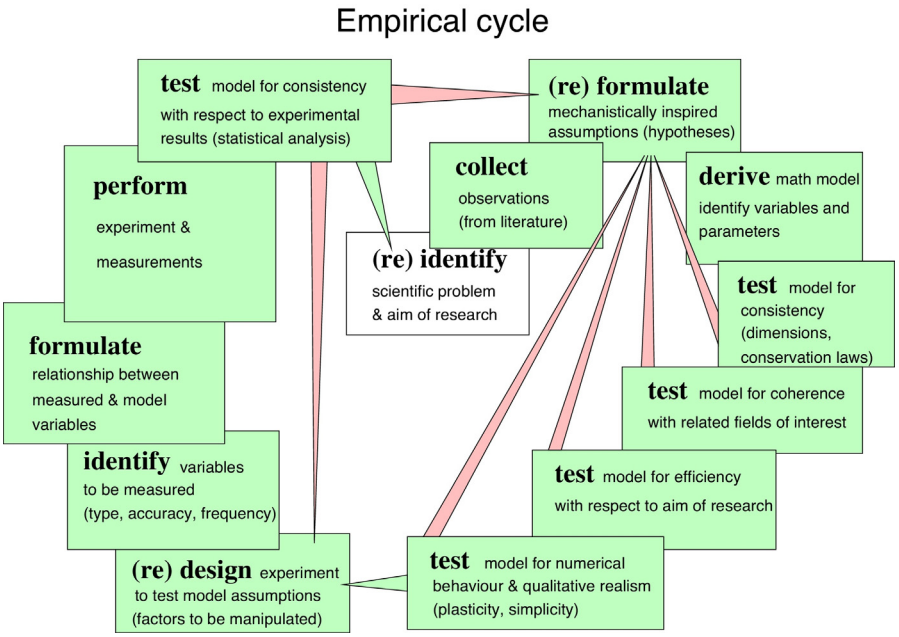

Fig. 1. The empirical cycle in the eyes of a theoretician starts with the formulation of the problem, using published work as source of inspiration for assembling a list of assumptions: the red arrows are followed in case of a bad result, the green ones otherwise. While experimentation is here only in one box, this does not mean that it is relatively little amount of work, but its significance fully rests on the rest of the cycle. The role of statistics is confined to the last step in the cycle. Many models don't need to be tested against data, since they already should have failed earlier tests in the cycle. More than half of the models that are published in the biological literature suffer from dimension errors and are, therefore, useless; some 80% of the manuscripts that I reviewed that were submitted for publication by journals also suffer from this. Given that nonsense models can easily fit data very well if they are sufficiently flexible, fitting data well is not the most important criterion for useful models. If the step from assumptions to the specification of the model is sufficiently lucid, a bad fit should lead to the assumptions that need replacement. Since the assumptions reflect insight, this can be seen as a step-up and, perhaps, the most useful role of models that are derived from assumptions. Such models are rare, however. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Please cite this article in press as: S.A.L.M. Kooijman, Models in stress research, Ecol. Complex. (2017), http://dx.doi.org/10.1016/j.ecocom.2017.07.006

Table 1
Some practical hints for starters in science.

- open a document with a unique label, your name, date, purpose; If your document is likely to contain quite some formulas, we suggest to use Latex, which is public domain
- make a list of assumptions (refer to literature items for support)
- make a list of symbols, variables *and* dimensions. Follow the mathematical rules for designing symbols; don't use names, like you will do in your computer code. Use different symbols for different dimension groups
- derive the equations, and insert any new assumptions or symbols that you need in the list. Include enough of these derivations into your document that you can understand them (much) later; check the consistency of your assumptions
- check dimensions before you proceed • write computer code from your written formulas; we suggest to use a fourth generation language, such as Octave, which is public domain. Insert your name and date in the computer code. Refer in your code to the document where you listed the formulas. Make a link between the variables in your code and the symbols in your document
- make sure that your code is doing what your formulas prescribe. If your code is not working yet, you still don't have a problem. Problems start as soon as your code is producing something, and you have to answer the difficult question whether or not that something relates to your model
- get a numerical feel for the potential behaviour of your model by making lots of graphs using different choices of parameters
- if you don't like the numerical behaviour of your model, don't start to change the code directly. Change assumptions first, re-do your derivations, then adapt the code (including the date of creation)
- make various simplifications of your model to see what the different elements of your model do. Learn to think in terms of families of models, rather than *the* model
- plan your experiment carefully, by imaging in detail what you are going to do with the results if you would have them. Will the results answer the questions that you have?
- think of calibrating your equipment before use; does the accuracy meet your requirements? Check mass and energy balances where possible.
- specify experimental conditions (sources of materials that are used, temperature, etc); label all experimental results carefully, you might want to re-use them at a much later moment; think of using a data-base
- fit your model to the data, and make a list of parameters, estimates and units. Never insert parameter values in models, because this obscures the units.
- compare the parameter values with your expectations, based on the literature
- what is your most promising next step? Discuss your results with colleagues. Consider contacting authors of papers that you read for your work; try to be a specific as possible in the questions that you will have

2.1. Part 1: from data to ideas

2.1.1. Identification

The identification of the scientific problem is the most crucial event in science and is even more interesting than a possible solution. Lack of progress in research can almost always be traced back to problems in the questions that were posed. Effort in polishing these questions, and make them as sharp and detailed as possible, is well spend and an effective time saver.

After identification of the scientific problem, the *empirical cycle* should start with the formulation of a set of assumptions, a *derivation* of a mathematical model from these assumptions, a sequence of tests on consistency, coherence, parameter sensitivity, and relevance with respect to the problem. See Fig. 1. Most models do not need to be tested against experimental data; they simply do not pass the theoretical tests.

2.1.2. Formulate assumptions

Assumptions are for me equivalent to hypotheses; some of them have more empirical support than others. The list of assumptions can usually be structured, some are more fundamental than others. Mass and energy conservation are examples of fundamental assumptions, so fundamental that one can argue that they should not count as assumptions. Yet being consistent with these assumptions is not the same as actively exploiting them, like DEB theory does. Strong and weak homeostasis are examples that certainly count as assumptions for metabolic modelling and strongly restricts possibilities for modelling. If one would not include them, however, I predict serious trouble later on in model testing. So they are examples of assumptions that one should not easily remove. The maternal effect, where DEB theory assumes that reserve density at hatching equals that of the mother at egg formation, is an example of the assumption that can easily be modified without much consequences for the rest of model structure. The better one can structure the list from general to particular, the more effective research becomes. It would be ideal to be able to test each assumption one by one, but this is rarely possible in practice.

2.1.3. Derive

The list of assumptions should be so specific that the step from assumption to the mathematical model is a technical one, not involving any new assumption. If this is not the case, the list of assumptions should be extended first.

2.1.4. Consistency

Proposition *X* is inconsistent with proposition *Y*, means that they cannot both be true. Models that are internally not consistent are meaningless, so they are useless. If different assumptions are directly contradictory, inconsistency is easy to detect. In many cases, however, this is much less easy. Inconsistencies come in many forms; lack of realism (meaning: a difference between measured data and model predictions for those data) is just one form (that comes in gradations).

An example of a model inconsistency that is apparently not so easy to detect is the log-logistic model for the cumulative number of offspring per female at a standardized exposure time as a function of the concentration of test compound. This very popular model (on which much of the environmental risk assessment in the world is based) has the form $N(c) = N_0(1 + (c/c_{50})^b)^{-1}$, where N_0 is the cumulative number of offspring in the blanc, c_{50} the so-called EC50 (50% Effective Concentration) and b a parameter that relates to the slope of the concentration-response curve. The bioassay with (female) daphnids is started with a number of concentrations, and a cohort of neonates in each concentration. The individuals develop and start to reproduce after about 7 days; the bioassay runs for 21 days. The inconsistency is revealed after the observation that reproduction rates tend to become constant after some time (after growth is ceased and internal concentration settled at some value). This means that the cumulative number of offspring eventually grows at a constant (concentration-dependent) rate. The implication is that, if the log-logistic model applies at some exposure time after the reproduction rates have stabilized, it cannot apply at any later exposure time. The assumption that the model applies at 21 days, together with the arbitrariness of this exposure period, in fact translates to the more stringent assumption that the model applies to all exposure times (even if it not used at other exposure times). This cannot be true, and the fact that the

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model fits empirical data is meaningless in the knowledge that other models fit these data too. Users of this model are probably not aware of the implicit assumptions about the reproduction process in a model that does not have time as an explicit variable. This type of problem rarely occurs if one starts from assumptions about mechanisms, rather than assuming the applicability of a model.

2.1.5. Dimensions

A dimension is an identifier for the physical nature of a quantity, see Table 2. A quantity of a particular dimension can be measured using several units; units determine the dimension fully. It is not necessary that all quantities in a model are measurable; the concept dimension is more general than the concept unit. Models that violate rules for dealing with dimensions are meaningless; it is a special case of inconsistency which frequently relates to errors in the translation of assumptions into a model. This does not imply that models that treat dimension well are necessarily useful models.

The elementary rules for manipulating dimensions are simple: addition and subtraction of variables are only meaningful if the dimensions of the arguments are the same, but the addition or subtraction of variables with the same dimensions is not always meaningful; meaning depends on interpretation. Multiplication and division of variables correspond with multiplication and division of dimensions. Simplifying the dimension, however, should be done carefully. A dimension that occurs in both the numerator and the denominator in a ratio does not cancel automatically. A handy rule of thumb is that such dimensions only cancel if the sum of the variables to which they belong can play a meaningful role in the theory. The interpretation of the variable and its role in the theory always remain attached to dimensions. So the dimension of the biomass density in the environment expressed on the basis of volume is cubed length (of biomass) per cubed length (of environment); it is not dimensionless. This argument is sometimes quite subtle. The dimension of the total number of females a male butterfly meets during its lifetime is number (of females) per number (of males), as long as males and females are treated as different categories. If it is meaningful for the theory to express the number of males as a fraction of the total number of animals, the ratio becomes dimensionless.

The connection between a model and its interpretation gets lost if it contains transcendental functions of variables that are not dimensionless. Transcendental functions, such as logarithm, exponent and sinus, frequently occur in models. pH is an example, where a logarithm is taken of a variable with dimension number per cubed length ($\ln\{\#l^{-3}\}$). When it is used to specify environmental conditions, no problems arise; it just functions as a label. However, if it plays a quantitative role, we must ensure that the dimensions cancel correctly. For example, take the difference between two pH values in the same liquid. This difference is dimensionless: $\dim(\mathrm{pH}_1 - \mathrm{pH}_2) = \ln\{\#l^{-3}\} - \ln\{\#l^{-3}\} = \ln\{\#l^{-3}\#^{-1}l^3\} = \ln\{\cdot\} = .$. In linear multivariate models in ecology, the pH sometimes appears together with other environmental variables, such as temperature, in a weighted sum. Here dimension rules are violated and the connection between the model and its interpretation is lost.

Another example of a model is the Arrhenius relationship, where the logarithm of a rate is linear in the inverse of the absolute temperature: $\ln \dot{k}(T) = \alpha - \beta T^{-1}$, where $\dot{k}$ is a rate, T the absolute temperature and α and β are regression coefficients. At first sight, this model seems to violate the dimension rule for transcendental functions. However, it can also be presented as $\dot{k}(T) = \dot{k}_\infty \exp\{-T_A T^{-1}\}$, where T_A is a parameter with dimension temperature and $\dot{k}_\infty$ is the rate at very high temperatures. In this presentation, no dimension problem arises. So, it is not always easy to decide whether a model suffers from dimension problems.

A further example of a model is the allometric function in body-size scaling relationships $\ln y(x) = \alpha + \beta \ln x$, or $y(x) = \alpha x^\beta$, where y is some variable, x has the interpretation of body weight, the parameter β is known as the scaling exponent, and α as the scaling coefficient. At first sight, this model also seems to violate the dimension rule for transcendental functions. Huxley introduced it as a solution of the differential equation $\frac{dy}{dx} = \beta \frac{y}{x}$. This equation does not suffer from dimensional problems, nor does its solution $y(x) = y(x_1)\left(\frac{x}{x_1}\right)^\beta$. However, this function has three rather than two parameters. It can be reduced to two parameters for dimensionless variables only. The crucial point is that, in most body size scaling relationships, a natural reference value x_1 does not exist for weights. The choice is arbitrary. The two-parameter allometric function violates the dimension rule for transcendental functions; uncertainty in the value of β translates into an uncertainty in the dimensions of α. Although this has been stated by many authors, the use of allometric functions is so widespread in energetics that it almost seems obligatory.

Variables are frequently transformed into dimensionless variables to simplify the model and get rid of as many parameters as possible. This makes the structure of the model more visible, and, of course, is essential for understanding the range of possible behaviours of the model when the parameter values change. The actual values of parameters are usually known with a high degree of uncertainty and they can vary a lot. *Buckingham's theorem* states that any relationship between m variables x_i of the form $f(x_1, \ldots, x_m) = 0$ can be rewritten as a relationship between $n = m - s$ dimensionless variables $y_i = h_i(x_1, \ldots, x_n)$ of the form $g(y_1, \ldots, y_m) = 0$, if the x's have s different dimensions. This shows that not only the number of parameters can be reduced by scaling, but also the number of variables.

2.1.6. Conservation laws

I discuss the application of conservation laws in DEB theory in some detail here, to illustrate that such a simple and widely known principle is basic to a radically different setup of models for metabolism, compared to the extensive literature on this topic. Despite the general attitude that conservation laws are self-evident, exploiting them to very open systems, such as living individual organisms, is not that self-evident at all.

Models that violate the conservation laws for mass, energy or time (or other conserved quantities) are rarely useful. It is a milder form of inconsistency, compared to dimension errors, for instance. (The physical conversion between mass and energy occurs on scales in space and time that is of little relevance to life on earth.) Conservation laws can frequently be written as a constraint on state variables x_j of a system in the form $f_i(x_1, \cdots, x_n) = 0$, where index i relates to the different conserved quantities (such as chemical elements, energy, etc).

Thermodynamics makes a most useful distinction between *intensive variables* – which are independent of size, such as temperature, concentration, density, pressure, viscosity, molar volume, and molar heat capacity – and *extensive variables*, which depend on size, such as mass, heat capacity and volume. Extensive variables can sometimes be added in a meaningful way if they have

Table 2
Symbols for frequently used dimensions.

·	dimensionless	#	number	t	time
l	length	m	mass	T	temperature

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the same dimension, but intensive variables cannot. Concentrations, for example, can only be added when they relate to the same volume. Then they can be treated as masses, i.e. extensive variables. When the volume changes, we face the basic problem that while concentrations are the most natural choice for dealing with mechanisms, we need masses, i.e. absolute values, to make use of conservation laws. This is one of the reasons why one needs a bit of training to apply the chain rule for differentiation.

DEB theory is not only consistent with conservation laws, but actively exploits conservation of chemical elements (C, H, O, N) to obtain respiration. Since all metabolic processes involve fluxes of these chemical elements, respiration, defined as the use of dioxygen, is by necessity a sum of several underlying processes. This approach is thus at odds with the idea that respiration would explain other metabolic features, such as growth and reproduction. This is very different from the huge amount of ecological literature, that models respiration as an allometric function of body weight, treats it as a quantifier for metabolic rate and seeks to explain almost all what an individual does with this metabolic rate (see section on meta-models). In a network of interrelating processes in an individual it is not that clear, at least to me, what THE metabolic rate actually means. While many have been wrestling to explain the empirical Kleiber's law Kleiber (1932), which states that respiration is proportional to biomass to the power 3/4, in the hope that this would explain all what an individual does, DEB theory provides the explanation, at least by numerical approximation, but does not give respiration that explanatory role.

Moreover, DEB theory exploits conservation of energy to obtain dissipating heat. This builds on the conservation of chemical elements, so a natural ordering exists: first mass then energy. We need both mass and energy balances to obtain entropy, as a third step, to fully specify what an individual is doing (Sousa et al., 2006). To my knowledge, DEB theory is the only one that can quantify entropy of living biomass. The existence of anearobic life (Bryant, 1991; Hochachka, 1980; Fenchel and Finlays, 1995) should already point to the limitations of using dioxygen use as quantifier for metabolic rate, and the existence of anearobic bacteria that consume heat, rather than produce it, should point to the limitation of using heat as such a quantifier.

DEB theory also exploits conservation of time to model behaviour. The most simple feeding module, for instance, partitions time into food searching and handling (sensu lato) as two sequential behavioural states of an individual. More advanced formulations further partition food handling into parts that must be sequential to searching (catching and ingestion) and parts that can be parallel to searching (digestion). By introducing more behavioural states, such as sleeping and social interaction, it becomes more subtle what states must be sequential and which can be parallel. DEB theory exploits the concept of Synthesizing Units to model these processes in a structured way (Kooijman, 1998, 2010).

Last, but not least, DEB theory exploits conservation of isotopes of the various chemical elements (Pecquerie et al., 2010; Kooijman, 2010). These conservation laws, in combination with the specification of the individual as a dynamic system, in fact specify the dynamics of isotopes. Isotope dynamics has been used, for instance, as support for the idea that structure, which is one of the components of biomass in DEB theory, is continuously broken down and resynthesized, with the accumulation of heavy isotopes over time as result.

2.1.7. Coherence

Coherence is the natural (logical) relationship between quantities. Assumptions should not contradict 'known' relationships in the context of the model. While consistency can only be judged for rather precise quantitative propositions, coherence is weaker and is judged for more qualitative propositions. Consistency mainly applies to the assumptions in direct relationship with the problem, coherence applies to the scientific neighbourhood of the problem in a wider context.

When growth of a female waterflea is modelled, for instance, and the reason for a limited maximum body size in the model is an increasing allocation to reproduction, it makes sense to remember that also male waterfleas exist that probably require a very different size control, even if male waterfleas are not topic of research. It is less likely that size control is different between the sexes. When growth of some species is modelled in a way that is very different from that of other species, it makes sense to remember that back in evolutionary history, these species have a common ancestor. How would that one grow? Such questions matter, even if modeling all species is not the topic of research.

The problem that everything depends on everything else in biology has strong implications for models that represent theories. When y depends on x, it is usually not hard to formulate a set of assumptions, that imply a model that describes the relationship with acceptable accuracy. This also holds for a relationship between y and z. When more and more relationships are involved, the cumulative list of assumptions tends to grow and it becomes increasingly difficult to keep them consistent. This holds especially when the same variables occur in different relationships. Moreover, the inclusion of more variables in the model also comes with an increase in constraints that relate to known properties of those variables.

2.1.8. Scales in organization

A more fundamental aspect to judge and evaluate coherence is the fact that the field of biology ranges from molecules, via cells, individuals, population, ecosystems to system earth. These levels of organization concern scales in space as well as in time. The words of Pascal still apply:

> The whole can only be understood in terms of its parts, but the parts can only be understood in the context of the whole.

Recent successes in molecular biology made holistic thinking less popular, however. Some workers seem to belief that soon they can explain all biology from the molecular level. The principle of *reduction* in science relates to the attempt to explain phenomena in terms of the smallest feasible objects. The hope for success can only be poor, however. Knowledge about technical details of engines in automobiles is extremely valuable for optimizing design, and reducing air pollution, but it is of little help to fight traffic jams. Similar relationships hold between molecular biology and ecology, these specializations focus on different space-time scales and deal with different processes that partially overlap.

Scales in space and in time are coupled in modelling because of problem of complexity (see next section). Models with a large time scale and a small spatial scale (or vice versa) will be complex, and complex models are not very useful. Using impressive computing power, it is feasible to model water transport in the earth's oceans, which seems to defeat the coupling of scales. The modeling of this physical transport, however, involves only a limited number of parameters (and processes), given the shape of the oceans' basins, explicit external wind forcing and information on planetary rotation. So model complexity might be less of a problem in this particular case.

2.1.9. Efficiency

A model should be *well-balanced in the level of details*. It makes little sense to construct a model for x, y and z that is very detailed in the relationship between x and (y, z), but not detailed at all in the relationships between y and z. Some plant growth models are very

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detailed in the module photosynthesis, for instance, where there is a role for all participating enzymes, but no detail at all on effects of nutrients, organic resources or water, environmental factors, not even on the dynamics of the amount of the various enzymes that partake in photosynthesis.

The avoidance of such unbalance by increasing the level of detail between *y* and *z* easily leads to complex models. All details should have a necessary function in the model, both conceptually and numerically; the principle of *parsimony* is to leave out less important details. What is a detail or an essential feature depends on the problem. The *efficiency* criterion boils down to the match in essential features in the model and in the problem, which makes that the model can be used optimally to find answers to the problem.

A major trap in model building is the *complexity* caused by a large number of variables. This trap became apparent with the advent of computers, which removed the technical and practical limitations for the inclusion of many variables. Each relationship, each parameter in a relationship comes with an uncertainty, frequently an enormous one in biology. With considerable labour, it is usually possible to trim computer output to an acceptable fit with a given set of observations. This, however, gives minimal support for the realism of the whole, which turns simulation results into a most unreliable tool for making predictions in other situations. The need for compromise between simplism and realism, makes modeling an art that is idiosyncratic to the modeler.

The only solution to the trap of complexity is to use nested *modules*. Sets of closely interacting objects are isolated from their environment and combined into a new object, a module, with simplified rules for input-output relationships. This strategy is basic to all science. A chemist does not wait for the particle physicist to finish their job, though the behaviour of the elementary particles determines the properties of atoms and molecules taken as units by the chemist. The same applies to the ecologist who does not wait for the physiologist. The existence of different specializations testifies to the relative success of the modular approach.

The problems that come with defining modules are obvious, especially when they are rather abstract. The first problem is that it is always possible to group objects in different ways to form new objects which then makes them incomparable. The problem would be easy if we could agree about the exact nature of the basic objects, but life is not that simple. The second problem with modules lies in the simplification of the input-output relationships. An approximation that works well in one circumstance can be inadequate in another. When different approximations are used for different circumstances, and this is done for several modules in a system, the behaviour of the system can easily become erratic and the approximations no longer contribute insight into the behaviour of the real thing.

In the first part of the empirical cycle, where the properties of models are analyzed, a powerful tool is to focus on the most simple models, and compare different models, where particular variables are included and excluded to study the effect of that variables. This can sometimes be done rather systematically, and families of models can be compared within a given framework. This is the happy hunting ground of *Mathematical Biology*, where model's simplicity allows the application of powerful mathematics.

2.1.10. Numerical behaviour

Before the realism of a model can be tested in a sensitive way, we need to study how the numerical behaviour of the model depends on the values of the variables and the parameters. Knowledge about the plasticity of the model is important in the estimation of parameter values, and in the best design of experiments. See the subsubsection on statistical testing against realism in the section part of the empirical cycle. The rescaling of variables to dimensionless quantities is a very useful tool to reduce the complexity of the model by eliminating parameters. A very useful strategy is to choose combinations of parameter or variables values that kick out a particular mechanism, and to compare the results with other choices of values. The contribution of each mechanism to the end result can be studied this way. It frequently happens that a few combinations of a number parameters (mainly) determine the numerical behaviour, rather than each parameter separately. This reveals opportunities to simplify the model. Odd behaviour of the model can point to undesirable interactions of assumptions, but more frequently to simple programming errors. If the odd behaviour is a genuine implication of assumptions, however, this is most helpful in the design of experiments to test its realism.

2.1.11. Qualitative realism

As last step in the first part of the empirical cycle, where we start thinking about what experiments and data we need to test all assumptions in the list, a few remarks on qualitative realism in general might help. If the qualitative behaviour if the model is bound to be realistic, we need to change one or more assumptions. Yet there is a more subtle aspect on qualitative behaviour. Model variables have been chosen on the basis of mechanistic criteria, irrespective whether they can be measured. Models that cannot be tested against experimental results are likely to be useless. Testability is in the twilight zone between the two sectors of the empirical cycle, but it comes in gradations. In most cases assumptions can be tested indirectly only, which involves other assumptions. This complicates the process of replacement of unrealistic assumptions in an attempt to find realistic ones, but this does not necessarily affect the usefulness of a model.

The weak homeostasis assumption in DEB theory, that specifies that structure and reserve of juveniles and adults grow (eventually) in harmony if food density remains constant, is made in the first place because if we don't make this assumption, I don't see any possibility to access the chemical composition and the masses of structure and reserve experimentally. We need the elemental frequencies of reserve and structure or access respiration in a DEB context, for instance, which is essential for energetics. We don't need any actual data to come to the conclusion that biomass components are hard to identify in absence of weak homeostasis, playing with thought experiments in mind will do. So the primary motivation for the weak homeostasis assumption in a DEB context is not realism, but to make the model testable.

If anywhere in this two-segment cycle appears the need to improve the model, it should not be changed directly, but the list of assumptions should be adapted, and the whole process should be repeated. It is a long and painstaking process, but sloppy procedures easily lead to useless results.

2.2. Part 2: from ideas to data

Now we have a model that follows from assumptions and survived all tests, we can start to judge its realism by testing it to data.

The variables that are easy to measure or those that will be used to test the model are not always those that should be state variables. An example is metabolic rate, which is measured as the respiration rate, i.e. dioxygen consumption rate or carbon dioxide production rate. The metabolic rate has different components, each of which follows simple rules. The sum of these components is then likely to behave in a less simple way in non-linear models. The composite nature of metabolic rate disqualifies it as explanatory variable in a model. The same holds for, for example, dry weights, which can be decomposed into structural biomass and reserve

Please cite this article in press as: S.A.L.M. Kooijman, Models in stress research, Ecol. Complex. (2017), http://dx.doi.org/10.1016/j.ecocom.2017.07.006

materials. A direct consequence of such partitioning is that experimental results that only include composite variables are difficult to interpret. For mechanistic models, it is essential to use variables that are the most natural players in the game. The relationship between these variables and those to be measured is the next problem to be solved, once the model is formulated.

So the second part of the empirical cycle starts with the formulation of *auxiliary theory* for how variables in the model relate to things that can be measured, the setup of adequate experiments and/or sampling and measurement protocols to test model predictions, the collection of the measurements, and statistical tests of model predictions against measurements. These tests could reveal that the protocols have been less adequate, and should be redesigned and executed; possible inadequacies should be detected in the auxiliary theory. So inconsistencies between data and model predictions not necessarily point to inadequacies in the model itself.

2.2.1. Experimental design

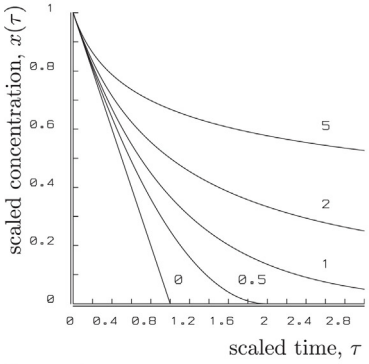
The art of experimental design fully rests on the prediction of the experimental results, and the choice of statistical procedures that will be used to evaluate the results. It is a form of reversed reasoning. The choice of experimental conditions, type of measurements to be made, details of sampling protocols to be used and other choices that have to be made can be motivated, for instance, by the minimization of the confidence intervals of particular model parameters that will estimated from the experimental results. A problematic aspect in explicit optimization of design is that models' parameters have to be known, while the experiment is usually done because they are not known. One has to rely on guesses, which might be in the wrong ball park. Moreover, the optimization of experimental design usually also involves constraints in terms of financial costs (including effort), ethical aspects, and availability of materials. The numerical analysis of the model (see previous section) is the main source of inspiration in the design of experiments.

2.2.2. Identification of variables to be measured

What can be measured and the precision of measurements depend on technical possibilities and financial costs that come with their own constraints. In the most straightforward and ideal situation the variables that occur in the model can be measured directly, without interference with the system (experimental artefacts). Practice is usually remote from this ideal situation.

The usual situation is that the variables that can be measured differ from those in the model, which calls for additional modelling for how the two sets are related. These models come with new parameters, and the numerical behaviour of this model (with variables that can be measured) should again be studied to optimize the design of the experiment and reduce the complexity

n-th order degradation



Dynamics

$$\frac{d}{dt}X = -\dot{\alpha}X^n$$

Solution

$$X(t) = \left(X_0^{1-n} - (1-n)\dot{\alpha}t\right)^{(1-n)^{-1}}$$

Special cases

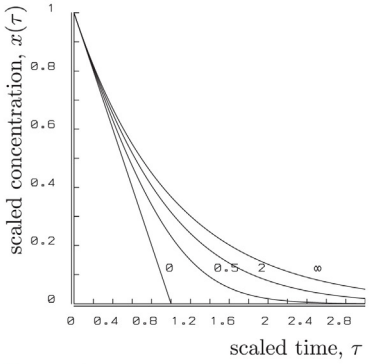
$$X(t) \overset{n=0}{=} X_0 - \dot{\alpha}t \quad \text{for } t < X_0/\dot{\alpha}$$
$$X(t) \overset{n=1}{=} X_0 \exp\{-\dot{\alpha}t\}$$

Scaled solution

$$x(\tau) = (1-(1-n)\tau)^{(1-n)^{-1}}$$

with $x \equiv \frac{X}{X_0}$; $\tau \equiv t\dot{\alpha}X_0^{n-1}$

Monod degradation



Dynamics

$$\frac{d}{dt}X = -\dot{b}\frac{X}{X_K + X}$$

Solution

$$0 = X(t) - X_0 + X_K \ln\{X(t)/X_0\} + \dot{b}t$$

Special cases

$$X(t) \overset{K \ll X_0}{=} X_0 - \dot{b}t \quad \text{for } t < X_0/\dot{b}$$
$$X(t) \overset{X_K \gg X_0}{=} X_0 \exp\{-\dot{b}t/X_K\}$$

Scaled solution

$$0 = x(\tau) - 1 + x_K \ln x(\tau) + (x_K + 1)\tau$$

with $x \equiv \frac{X}{X_0}; \tau = \frac{t\dot{b}}{X_K + X_0}; x_K = \frac{X_K}{X_0}$

Fig. 2. The n-th order model for biodegradation of a compound X during time t is much more flexible in its morphology as a function of parameter values then the Monod model, while both models have three parameters (start concentration X_0, a rate parameter, $\dot{\alpha}$ or $\dot{b}$ and a shape parameter: the order n or the saturation constant X_K). Both models give identical $X(t)$ curves if $n=0$ and $X_K \to 0$ and if $n=1$ and $X_K \to \infty$. While all possible shapes of curves can be scaled between these two boundaries for the Monod model, many other shapes are possible for the n-th order model. This means that observations better determine the parameter values of the n-th order model, but that a good fit gives less support, compared to the Monod model. Moreover, the n-th order model suffers from dimension problems if n is not an integer, and has a more complex link withmechanisms, if any.

Please cite this article in press as: S.A.L.M. Kooijman, Models in stress research, Ecol. Complex. (2017), http://dx.doi.org/10.1016/j.ecocom.2017.07.006

of the model. It frequently happens that the experiment is not a single experiment, but a set of possible very different experiments, in which different variables are measured. Some of these experiments require experimental pilot studies before the "final" experiment can be set up in an optimal way.

It is physically impossible to measure something, without interference with the system. The amount of disturbance must be evaluated in one way or another, usually by comparing results of experiments in which the disburbance is of a different nature.

Before actually performing costly (and/or time consuming) experiments it can be very useful to fake the possible measured values first, and complete the full cycle of statistical testing, using these faked values. It might be unrealistic to expect that the experiment results can possibly be satisfying, and that more effort should be invested in further optimizing the design or in the setup of alternative experiments.

2.2.3. Experimentation

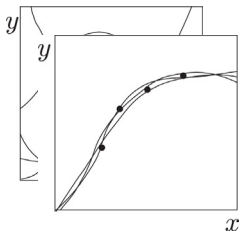
This is not the right place to focus on the many aspects of experimentation, and e.g. procedures for calibration of measurement devices. Similar to modelling, testing mass and energy balances can be a very useful tool to check experimental results on the consistency in ways that hardly depend on modeling details.

Needless to say is that the source of data not only can come from new experiments or measurements, but also from existing ones, e.g. as reported in the literature. The specification of experimental details can be a matter of concern, however, especially in modern literature, where the focus is on reduction of the size of papers. Reported data are frequently incomplete; many papers have been written on the body growth or reproduction of some species without mentioning the temperature, for instance, which is essential for interpretation and comparison. Even more troublesome is that these measurements, taken from the literature, probably have been done by people with a very different idea in mind and that this still fits the needs is sheer coincidence. This is because the context of measurements typically matters a lot and reported aspects of the context depend on the ideas in mind.

2.2.4. Statistical testing against realism

Model predictions for measurements (or experimental results) will always differ from these measurements because models are idealisations, and repeated measurements are not identical. Whether or not a given difference is large or small depends on the specified problem. The judgement can usually be formalized in a statistical procedure for that problem, called a test, that can result in a judgement "unacceptably large", in which case the model failed the test against experimental data. A realistic model is a model that predicts measured values with a small difference only. The measurements then support the model, and give no reason to change or replace assumptions. Such a support can, however, never prove that the assumptions are right.

The amount of *support* that a successful test of a model gives depends on the model structure and has an odd relationship with the ability to estimate parameters: the better one can estimate parameters, the less support a successful test of a model gives. This is a rather technical but vital point in work with models. This can be illustrated with a simple model that relates y to x, and which has a few parameters, to be estimated on the basis of a given set of observations $\{x_i, y_i\}$. We make a graph of the model for a given interval of the argument x, and get a set of curves if we choose the different values of the parameters between realistic boundaries. Two extremes could occur, with all possibilities in between: The curves have widely different shapes, together filling the whole x, y-rectangular plot. Here, one particular curve will probably match the plotted observations, determining the parameters in an accurate way, but a close match gives little support for the model; if the observations were totally different, another curve, with different parameter values, would have a close match.



The curves all have similar shapes and are close together in the x, y-rectangular plot. If there is a close match with the observations, this gives substantial support for the model, but the parameter values are not well determined by the observations. Curves with widely different parameter values fit equally well.

Two alternative models for biodegradation, with the same number of parameters, illustrate both situations in Fig. 2. Of course, the choice of the model's structure is not free; it is dictated by the assumptions. So, testability is a property of the theory and nice statistical properties can combine with nasty theoretical ones and vice versa. It is essential to make this distinction.

An increase in the number of parameters usually allows models to assume a much wider range of shapes in a graph. This is closely connected with the structural property of models just mentioned. So a successful test against a set of observations gives little support for such a model, unless the set includes many variables as well. A fair comparison of models should be based on the number of parameters per variable described, not on the absolute number.

2.2.5. The role of statistics: stochastic versus deterministic models

Statistics is developed to analyse the link between data and model, so *the* framework to use in judging realism of models. Yet, I frequently meet cases of improper use in biological research, which motived me to make some general remarks about the nature of scatter, especially in biology.

Observations show scatter, which reveals itself if one variable is plotted against another. It is such an intrinsic property of biological observations that deterministic models should be considered as *incomplete*. The mechanism behind scatter is frequently the effect of a large number of factors that influence the result, but are not modeled explicitly. Think, for instance, about modeling the outcome of throwing a dice. A complex deterministic model can (in principle) predict the outcome, when the forces, the trajectory in the air, the tumbling and bouncing is modeled in great detail, including the many imperfection's of dice and table. A very simple stochastic model (with the six possible outcomes having equal probability) usually works better become most parameters of the deterministic model are not known, and the process of throwing cannot be controlled in sufficient detail. This example reveals that it should usually be possible to reduce scatter (deviations between measurements and predictions by deterministic models) either by modeling more factors, or by excluding the scatter inducing factors experimentally.

Only *complete models*, i.e. those that describe observations which show scatter, can be tested. The standard way completing deterministic models is to add 'measurement error'. The definition of a measurement error is that, if the measurements are repeated frequently enough, the error will disappear in the mean of these observations. Such models are called regression models: $\underline{y}_i(x_i) = f(x_i|\text{pars}) + \underline{\varepsilon}_i$. They are characterized by a deterministic part, here symbolized by the function f, plus a stochastic part, $\underline{\varepsilon}$.

Please cite this article in press as: S.A.L.M. Kooijman, Models in stress research, Ecol. Complex. (2017), http://dx.doi.org/10.1016/j.ecocom.2017.07.006

The latter term is usually assumed to follow a normal probability density, with mean 0 and a fixed variance, which is one of the parameters of the model.

The interpretation of scatter as measurement error originates from physics. It is usually not realistic in biology, see Bedaux and Kooijman (1994), where many variables can be measured accurately in comparison with the amount of scatter. The observations just happen to differ from model expectations. When the scatter is large, the model is useless, despite its goodness of fit as a stochastic model. A realistic way of dealing with scatter is far from easy and usually gives rise to highly complicated models. Modelers are frequently forced to compromise between realism and mathematical over-simplicity. This further degrades the strict application of goodness of fit tests for models with unrealistic stochastic components.

3. Dynamic systems

Most models of biological systems are phrased in the context of dynamic systems, since life is a process. This especially applies to stress research, since stress is essentially a deviation from unstressed behaviour. For stress by chemical compounds, exposure to the compound is key, and the response is very much time dependent.

The idea behind the concept of a dynamic system is simple in principle. A system is based on the idea of *state variables*, which are supposed to specify completely the state of the system at a given moment. Completeness is essential. The next step is to specify how the state variables change with time as a function of a number of *inputs* and each other. The specification usually takes the form of a set of *ordinary differential equations* (ode' s)

$$\frac{d}{dt}x = f(x|\theta) \quad \text{for } x = (x_1, \ldots, x_n) \tag{1}$$

which have parameters θ, i.e. constants that are assumed to have some fixed value in the simplest case. Usually this specification also includes a number of *outputs*. The set of differential equations fully specifies the dynamics of the system in combination with the specification of the system at the start, $x(0)$, or at some moment in time, $x(t_1)$. Finding the states of the system as a function of time is called a *initial value* problem, of a *boundary value* problem, respectively.

Parameters are typically constant, but sometimes the values change with time. This can be described by a function of time, which again has parameters that are now considered to be constant. For instance, parameters that have the interpretation of physiological rates depend on temperature; therefore, they remain constant as long as the temperature does not change. If the temperature does change, then the parameters do as well. Heat, however, is generated as a side product of metabolism. In ectotherms, i.e. animals that do not heat their body to a constant high temperature, heat production is low, because of their usually low body temperature. The body temperature usually follows that of the environment, and can thus be treated as a function of time. The situation is more complex in developing birds, which make the transition to the endothermic state some days after hatching. The hatchling's temperature is high, because of brooding; therefore, metabolism and heat production are also high. In addition, the young bird starts to invest extra energy in heating. Here, the state variables of the system interfere with the environment, but not via input; this means that the body temperature must be considered as an additional state variable.

Choosing the state variables is the most crucial step in defining a system. It is usually a lot easier to compare and test alternative formulations for the change of state variables, than different choices of state variables. Models with different sets of state variables are hardly comparable, both conceptually and in tests against data. Statistics basically deals with parameter values, and is of little use when comparing the goodness of fit of models that differ in structure.

3.1. Constraints on dynamics

Mass, energy and other conserved quantities pose constraints on the possible behaviour of dynamic systems. The explicit use of these constraints forms a powerful tool in the specification of the dynamics of the system. If the states are appropriately specified, these k constraints on $x_1, \ldots x_n$ can be written in the form $g_i(x) = 0$, for $i = 1, \ldots, k$. If all x_i represent masses, the functions g_i take the form $\sum_{ij} w_{ij} x_j = 0$, where w_{ij} are fixed weight coefficients. The system can be reduced to $n - k$ variables, and we have to find the remaining k variables from the k constraints. If the system is not reduced, the Jacobian matrix of the system will have k eigen values equal to zero at any point in time. This might hamper the application of software for the analysis of asymptotic properties of the system.

3.2. Feedback

Systems can behave in ways that cause an amplification (positive feedback) or a reduction of that behaviour, called *negative feedback*. A growing population of organisms is likely to grow faster because more organisms partake in reproduction (positive feedback), but they exhaust their resources sooner (negative feedback). The notion "feedback" originates from engineering, where systems are constructed. In biology, where systems are usually given, and many components of the system work in opposite directions, the notion is less operative.

3.3. General systems theory

On a historical note, von Bertalanffy was an advocate of the application of general systems theory in biology (von Bertalanffy, 2011), which makes intensive use of dynamic systems. Like typical for his time, he thought of growth as resulting from the difference between anabolic and catabolic processes (von Bertalanffy, 1938, 1940), not in terms of inputs and outputs of the individual with mass conservation, as is the basis of DEB theory. What is presently known as the von Bertalanffy growth equation was proposed 18 years earlier by Pütter (1920), as acknowledged by von Bertalanffy himself, where change in length is proportional to the different between actual and ultimate length: an interplay between surface area and volume of an isomorph (an organism that does not change in shape during growth). Pütter had an explanation for why he took the 'von Bertalanffy growth rate' to be inversely proportional to ultimate length: a growth-inhibiting compound appears at a rate proportional to the decay (volume) and disappears a rate proportional to the supply of anabolic substrate (surface area). The comments on DEB3 (Kooijman, 2016, Section 2.4) give a more detailed account. Notice that the 'von Bertalanffy growth rate' is linear in ultimate length in DEB theory, so very close to Pütter's idea, and DEB theory also lets compounds affect parameters. Von Bertalanffy modified Pütter's ideas and replaced surface area and volume by allometric functions of body weight, but this modification, somehow, did not associate with his name. Huxley, who introduced these functions in biology (Huxley, 1932), was well aware of the problem that is associated to allometric functions and stressed that they were descriptive functions only; a wise warning that went lost in time. These functions did a lot of harm in the further development of biology as a discipline.

Please cite this article in press as: S.A.L.M. Kooijman, Models in stress research, Ecol. Complex. (2017), http://dx.doi.org/10.1016/j.ecocom.2017.07.006

4. Metabolism

After this general introduction on the role of models in research, and on the art of modelling, I now briefly sketch modelling issues concerning stress. Since such stressors modify the (physiological) behaviour of organisms, we first need to focus on the situation without stress, to expose where the handles exactly are that stressors can potentially modify to affect metabolism. The general idea is that effects of stressors translate to changes in parameter values.

DEB theory is designed to do exactly that. The task of this text is, however, not to explain its setup in detail, but to sketch its scientific setting. The motivation of this presentation is to explain why the standard DEB model is not just one of many alternative possibilities and that it follows naturally from limited number of first principles: why we need a pool-approach, as opposed to a biochemical one, and why we need a number of homeostasis concepts in a pool approach. Most of the model structure directly follows from these homeostasis concepts; the dynamics of reserve follows uniquely from weak homeostasis, for instance, and other key concepts, such as the κ-rule and maturity maintenance basically follow subsequently from consistency arguments, (Lika and Kooijman, 2011). This leaves very little room for alternative model formulations.

But the proof of the pudding is in the eating: the standard DEB model, together with minor modifications of it, turned out to fit a wide variety of data very well, on energetics and life history of 683 animal species (sampling date 2017/04/17) with a mean relative error of less than 0.1, see Curators of the AmP collection (2017). For each species, a variety of data sets could be found such that all core parameters could be estimated, see Table 3, allowing us to compare species on the basis of parameter values. The species are from all large animal phyla and all chordate orders, including most species that are popular in ecotoxicity testing. Their body sizes cover the full size range among animals, that of hairy back and blue whale differ by 16 orders of magnitude. This also applies to their life spans, that of rotifer and Greenland shark differ by more than 4 orders or magnitude. The range of habitats in which they live, and their range of positions in the trophic chain, is close to maximum possible as well. This performance is, and I think will remain, unchallenged. The implication is that the parameters of the standard DEB model are *the* natural handles for effects of stressors.

4.1. Biochemical vs pool approaches

I see two possible approaches for the quantification of metabolism, the biochemical and the pool approach, which are to some extend complementary. The chemical approach follows particular chemical compounds. Since the actual number of different chemical compounds in an organism is very large indeed, we are forced to classify compounds in a few important ones that we choose to follow and the rest that we need to leave alone. Since important compounds can be transformed to unimportant ones, and vice versa, this approach is hard to combine with mass (and energy) balancing. The pool approach delineates pools of metabolites, defined as mixtures of chemical compounds that can change in mass, but not in chemical composition. The general idea is that biomass is partitioned in a very limited number of such pools, just enough to allow this idealised situation, called strong homeostasis, to be approximately realistic. This approach is easy to combine with mass balancing, but obviously suffers from the idealisation step which might not hold in detail and also not under all circumstances. Most metabolic models in ecology follow the pool approach, and in fact delineate just a single pool: biomass. They cannot handle changes in the composition of biomass. DEB theory delineates reserve and structure, and the reproduction buffer in adults, as pools with the consequence that weights have contributions from several pools and are, therefore, less easy to interpret. This can handle particular changes in the composition of biomass.

4.2. Static vs dynamic approaches with production vs assimilation models

Most metabolic models in ecology can be classified as Static Energy Budgets (SEBs). They take a mental snapshot of fluxes of compounds to and from an individual, and model their fate with what is known as production models: food intake is (mentally) converted to energy, losses in the form of faeces, urine and respiration are subtracted, and the remaining flux is allocated to production: growth and/or reproduction. SEBs have problems with the embryo stage, since this stage has no assimilation (no food intake), and is ignored. When individuals are followed in time, a series of such snapshots are taken and these fluxes become time-dependent. A fundamental problem with this approach is in the interpretation of respiration as loss. Part of respiration relates to overhead costs for growth and reproduction, so to production: the bookkeeping is not correct. To separate allocation to growth and reproduction into overhead and net fixation, we need a dynamic approach, not a static one.

A Dynamic Energy Budget (DEB) follows the individual through its complete life cycle (and so life stages): from the start of development of an embryo till death; it views the individual as a dynamic system that changes over time. Since eggs/seeds decrease in (dry) mass over time, but increase in respiration, DEBs need to partition biomass in at least 2 pools. Foetal development is a variation on egg development, where the foetus receives resources from the mother during development. DEB theory assumes that the only difference between an embryo and a juvenile is assimilation, and extreme point of view that fits data very well. It also assumes that the difference between a juvenile and an adult is that a juvenile does not allocate reproduction, and an adult not to maturation.

SEBs typically delineate just a single pool, biomass, and several more problems show up: Urine production is not (only) directly coupled to food intake, nor is respiration, growth and reproduction. Many insects lay eggs as imago's, for instance, but don't eat: allocation to reproduction already occurred in the larval stage, when they had a different body size. Many fish and molluscs spawn in periods when little food is around: allocation to reproduction occurred much earlier.

The standard use of production models naturally leads to the idea that growth directly competes with reproduction in terms of allocation. Not so in DEB theory, where somatic maintenance competes with growth and reproduction with maturity maintenance. Growth and reproduction compete only indirectly, via the κ-rule: a fixed fraction of mobilised reserve is allocated to somatic maintenance plus growth the rest to maturity maintenance and maturation (before puberty) or reproduction (after puberty). Waste-to-hurry is the phenomenon that assimilation and (somatic) maintenance, growth (to a small asymptotic body size) and reproduction are all increased (Kooijman, 2013). It is typically seen in species that live on substrates that are abundant during a short period. This combination follows naturally from the κ-rule, but is much more difficult to understand in the context of production models, since the system can only respond to the difference between assimilation and maintenance. Yet it explains the existence of what is since 1963 known as futile cycles in biochemistry (Qian and Beard, 2006; Stein and Blum, 1978; Steinberg, 1963); all species have this ATP-decaying cycle (the enzymes and genes that are involved are known), and some use it intensively, but its function remained elusive. I mention this

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Table 3
The standard DEB model, which is the simplest non-degenerated model in the context of DEB theory, has the parameters in a time-length-energy frame:

Symbol	Units	Name	Function
T_A	K	Arrhenius temperature	changes in temperature
$\{\dot{F}_m\}$	$dm^3/d.cm^2$	specific searching rate	changes in food density
κ_X	–	digestion efficiency	food quality
$\{\dot{p}_{Am}\}$	$J/d\,cm^2$	specific maximum assimilation	metabolic capacity
$\dot{v}$	cm/d	energy conductance	reserve mobilisation
κ	–	allocation fraction to soma	allocation
$[\dot{p}_M]$	$J/d\,cm^3$	vol.-spec. somatic maintenance	structure turnover, movement
$\{\dot{p}_T\}$	$J/d\,cm^2$	surf.-spec. somatic maintenance	heating, osmotic work
$[E_G]$	J/cm^3	specific costs for structure	reserve-structure conversion
$\dot{k}_J$	1/d	maturity maintenance rate coeff.	information maintenance
κ_R	–	reproduction efficiency	conversion of reserve to eggs
E_H^b	J	maturity at birth	embryo-juvenile transition
E_H^p	J	maturity at puberty	juvenile-adult transition
h_a	$1/d^2$	Weibull aging acceleration	aging
s_G	–	Gompertz stress coefficient	aging acceleration

example to illustrate that the choice of model structure typically not depends on differences in goodness of fit between models, but on qualitative behaviour. Another motivation is that we found that no effect concentrations of pesticides strongly decrease for increasing specific somatic maintenance of species, see the Section on Meta-models, and observe that agricultural techniques strongly select for species that follow the waste-to-hurry strategy, by offering abundant food, but during a short period only. Further research (not yet published) revealed that the no effect concentration does not depend on specific somatic maintenance for a number of other groups of compounds, suggesting that species with elevated somatic maintenance share a set of biochemical pathways that make them sensitive for pesticides. This is one of the very rare examples where sensitivity for a particular class of chemical compounds can directly be linked to physiological properties, and in this case also to ecological performance.

4.3. Potential handles for stress

Now that models for metabolism has been briefly discussed, I need to become a bit more detail for DEB theory to expose the handles for metabolic stress. If one would choose for a different metabolic model, stress will also be modelled differently by necessity. It is not possible to model stress without modelling metabolism first.

As mentioned before, biomass is partitioned into reserve and structure (and the reproduction buffer for adults), but it also has the state variable maturity to specify metabolic switches. Maturity has no mass or energy in DEB theory, just information, which is quantified by the cumulative investment of reserve the create it. This can be expressed in energy of mass, but the investment itself dissipates. Maturity maintenance is proportional to maturity, likewise somatic maintenance is proportional to structural volume. Somatic maintenance and also have a component that is linked to (structural) surface area for heating or osmotic work, but this investment depends on the (changing) environment.

The standard DEB models has 15 core parameters, see Table 3, i.e. handles for stressors. The reproduction buffer handling rules, that are used to convert the reproduction buffer to eggs, can have additional parameters. Thanks to strong homeostasis, the parameters can also be expressed in a time–length–mass frame using simple conversions.

After exploiting conservation laws, the most typical elements of DEB theory are five forms of homeostasis: the ability to run metabolism as independently as possible from the (varying) environment. The first 2 forms of homeostasis apply to all species, the other 3 only to some of the species:

- : pools (reserve, structure) do not change in composition
- : after birth, pools grow at constant food density such that their ratio remains constant
- : shape remains constant during growth
- : body temperature is regulated at a constant level, independent of the environment
- : food intake is as independent as possible from food availability (relates to supply-demand spectra Lika et al., 2014a,b)

It is clear that all pool models must make use of the concept strong homeostasis, since its one-one link with the definition of the concept pool. Weak homeostasis is required to access the chemical composition and the size of the pools experimentally; without this concept the identification of pools will be extremely difficult, if not impossible, which turns the model in being empirically untestable. The relevance of structural homeostasis is that food intake is taken proportional to surface area, and somatic maintenance to volume. Although the standard DEB model assumes structural homeostasis, this assumption is not part for DEB theory in general. If it applies, food intake is proportional for squared length, while somatic maintenance is proportional to cubed length. Length in the dimension of the parameter energy conductance, $\dot{v}$, is in fact the ratio of volume and surface area. Organisms can accelerate their metabolism by changing their shape (Kooijman, 2014; Lika et al., 2014a,b).

5. Stress

Now the handles for stress have been discussed, various forms of stress can be introduced as deviations from the eco-physiological behaviour without stress. Since, world wide, most research is done on chemical stress, it is discussed in somewhat more detail the next section.

5.1. Food stress

Resources (food) can be inadequate in quality and amount. The number of different resources that are taken up from the environment matches the number of reserves that are delineated and stoichiometric constraints apply to convert resources to reserves. Animals are organisms that live of (products of) other organisms, so complex in chemical composition, which is the reason why a single reserve is sufficient for most purposes. Any inadequacy of food quality then results into a lower digestion efficiency and translates into an inadequacy of amount of food. This also affects faeces production and mineral (CO_2, H_2O, O_2, NH_3) fluxes that are linked to assimilation. DEB theory has no

assumptions on respiration, which is frequently taken to quantify metabolic rate, and gets respiration by closing the mass balances for chemical elements, where the mineral fluxes play a key role. DEB theory respects and exploits balances of energy and chemical elements, but also stoichiometric constraints on chemical transformations. This is why all inputs to and outputs from the individual matter in a metabolic context, and how they change during ontogeny.

Most animals eat meals and starve in between. The gut/stomach system serves to smooth out these fluctuations in food intake on a short time scales, reserve does that on a longer time scale. Mild starvation affects reserve only without any adjustments. When starvation continues and reserve, and so reserve mobilisation decreases such that maintenance costs can no longer be paid from mobilised reserve, adults with a reproduction buffer can use this buffer to cover maintenance costs. Prolonged starvation leads to rejuvenation (reduction in the level of maturity) and shrinking (reduction in the amount of structure). Rejuvenation affects the hazard rate, shrinking is possible up to a maximum fraction when death by starvation kicks in. Supply-species can handle starvation better than demand-species, see Lika et al. (2014a,b).

5.2. Temperature stress

The Arrhenius relationship generally quantifies affects of temperature on rate parameters well within a limited temperature range. Near these boundaries metabolism is (much) slower, which is modelled by assuming that enzymes have one active and two (high and low temperature) inactive states; conversion to these inactive states again follows the Arrhenius relationship, resulting in a 5 parameter temperature module (Sharpe and DeMichele, 1977; Schoolfield et al., 1981; Kooijman, 2010).

This deviation from the Arrhenius relationship quantifies what happens with metabolism during torpor (including hibernation); low temperature in temperate and (ant)arctic climates or high temperature in (sub)tropical climates (dry season) typically coincides with periods when food is scarce.

Within a limited temperature range, endotherms can fine-tune their behaviour and insulation to control their body temperature with hardly any effects on the energy budget: the thermal neutral zone. For higher temperatures they might increase evaporation (see under water stress) and for lower temperatures their surface-area specific somatic maintenance cost, $\{\dot{p}_T\}$.

Effects of temperature on metabolism frequently play an important role in the geographical distribution of a species; for terrestrial species, thermal balances need to be made for detailed prediction (Kearney, 2011, 2012, 2013; Kearney et al., 2010, 2012).

5.3. Aging and radiation

Aging is modelled by linking the use of dioxygen or other free radicals to the formation of damage-inducing compounds (think e.g. of modified mitochondrial DNA), which generate damage compounds (think e.g. of modified proteins) at constant rate (van Leeuwen, 2003; Kooijman, 2010). The hazard rate that is associated with aging is proportional to the density of damage compounds; hazard rates can have a variety of contributions (predation, accidents, illness, extreme starvation). Damage-inducing compounds can also generate more damage-inducing compounds, if the Gompertz stress coefficient is positive; this construct is realistic for endotherms. This way of modeling aging captures the effects caloric restriction on aging very well (van Leeuwen et al., 2002; Kooijman, 2010).

Although parameter values other than the hazard rate can also be linked to the concentration of damage compounds, this is not done in the standard DEB model. This can be done to model post-reproductive periods, for instance. Gradual effects of ageing can also be linked to the residence time of molecules in reserve, which increases with length, with the idea that the 3D configuration of proteins can change in time, which affects their functionality.

Radioactive and UV radiation can also generate free radicals and enhance the ageing process by increasing h_a. Moreover free radicals can induce tumours; their growth can be modeled as a new structures with their own maintenance costs, that compete with the original structure (van Leeuwen and Zonneveld, 2001; van Leeuwen, 2003; van Leeuwen et al., 2003; Kooijman, 2010). This way of modeling tumour growth captures the effects of caloric restriction and the host-tumour interaction very well; a tumour that is induced in a juvenile follows a very different trajectory compared to that in a fully-grown adult. Many different types of tumours exist, and the general idea is that they can be compared on the basis of parameter values.

5.4. Stress by hypoxia, water and osmosis

The standard DEB model assumes that food availability is the only restriction on metabolism. If dioxygen is in short supply as well, it counts as a second limiting substrate and multiple resource models must be used, with stoichiometric constraints on conversions. Many species can sport fermentation, which involves fermentation products. DEB theory has no problems with its quantification (Kooijman, 2010), but alternative fermentation products and the gradual transition between aerobic and anaerobic metabolism involves extra parameters.

The standard DEB model assumes that the water content of food, reserve and structure is constant, and quantifies the excretion of metabolic water via the conservation of chemical elements. Water losses through evaporation in terrestrial species can be linked to respiration and via surface area through skin or other surfaces and depends on humidity and temperature (which can vary in time). The general idea is that these losses are compensated by drinking. Water stress can be quantified as the ratio of the normative and actual water content and death kicks in when it exceeds a threshold level. The normative water content is the water content in absence of water stress, but it can depend on environmental factors.

Effects of osmosis are modeled in the same way as surface-linked body temperature in endotherms: outside a given range of ionic strength, (aquatic) organisms can invest in surface-linked somatic maintenance to solve problems. If not sufficient, they die.

5.5. Stress by chemical compounds

Toxic effects are typically evaluated in a number of simple standardized toxicity experiments, but the reason to do these experiments is to predict potential effects of man-made chemicals in the environment, which involves quite a list of complicating factors. Moreover organisms themselves produce compounds that affect other organisms, think for instance of the moss *Sphagnum* or tree leaf litter, which decrease the pH or the weed *Chara*, which increases the pH, or of dinoflagellates and fungi that can produce toxicants, with dramatic effects on other organisms. My discussion of stress by chemicals is in the wider context and I see it as an important aspect of the evolution of life on earth.

Effects of chemical compounds are linked to their concentration (or density) in the body (Kooijman et al., 2009a,b). Three ranges are delineated for each target parameter: 'too little', 'enough' and 'too much'. Some of these ranges can be zero; cadmium availability, for instance, cannot be 'too little' for any target parameter. In the enough-range, compounds have no effect on metabolism, which is

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why there is no need to think about effects of chemical compounds if there is no need for this. The boundaries of the enough-range are called the internal no-effect-concentrations. Since the 'too little'-range for many human-made chemicals is zero, THE internal no-effect-concentration is frequently taken to be the upper boundary, unless specified otherwise.

Mutagenic compounds have similar effects as free radicals, as discussed in the subsection on aging, including the induction of tumours. Their too-little- and enough-ranges are typically zero. Teratogenic compounds affect development in a qualitative way that is compound- and species-specific. These effects must be modeled case by case.

Most chemical compounds affect metabolism via the parameters. Chemicals are classified as narcotic, or endocrine disrupting, but these classifications not necessarily link to the various DEB parameters that are effected. Since most practical interest is in small effects, the change in parameter value can be taken proportional to the concentration that is too little or too much, so positive part of the difference between the actual and no-effect concentration. The arguments rests on a Taylor approximation; small changes in parameter values can have large changes in endpoints, but DEB theory takes care of these non-linearities. Lethal effects are modeled via the hazard rate as target parameter. Independent effects on survival correspond with additive terms in the hazard rate, so effects of aging, background mortality and chemical compounds on survival all have adding contributions to the hazard rate. Sublethal effects typically, but not always, occur at much lower concentrations in the body. The no-effect-concentrations for all parameters can be ordered, and the focus is typically on the most sensitive parameter only, which reduces the complexity of the problem substantially for small effects.

Most chemicals in a living body transform in one way or another, all chemical species can have effects. The simplest way to deal with this complexity is to assume that all concentrations of these chemical species are proportional to each other. This is not always realistic and the dynamics of these chemical species must then be followed explicitly. The next simplest approach is to ignore interactions between these chemical species. Interactions between two chemical species can generally be modeled as an additive term that is proportional to the product of their (internal) concentrations. With more species, we take all possible combinations of two pairs on the basis of the Taylor-approximation for multivariate non-linear functions, which is supposed to hold for small changes in parameter values.

Since mixtures involve several chemical compounds, the best strategy is to quantify them as moles, not grams, since each molecule can have an effect, not each gram. Although most papers in ecotoxicology compare effects of compounds on the basis of grams, this is actually a very strange habit that should be ceased as soon as possible.

Since effects are linked to internal concentrations, processes that affect these concentrations indirectly affect effects. We need to consider the various uptake and elimination routes, as well as account for dilution by growth and metabolic transformation. Lipophylic compounds 'prefer' to be in lipids and if reserve is more rich in lipids than structure, reserve density affects uptake. As long as compounds are in a lipid matrix in biomass, they typically have hardly any metabolic effect, but effects might show up as soon as lipids are used for metabolism. Excretion is typically only possible when compounds are not in the lipid matrix; they are (again typically) first transformed to hydrophilic compounds before they are excreted. These hydrophylic metabolites are generally more toxic than the original lipophilic compound, but their concentrations can be low. Elimination via reproduction (eggs, sperm) can be important, particularly for lipohilic compounds via eggs. Elimination via active excretion can occur via skin, gills and gut and can depend on metabolism.

Both (direct) elimination and uptake rates are proportional to surface area of the individual, which is why the internal concentration satiates faster in small-bodied individuals, compared to large-bodied ones. Uptake can be directly through the skin, via the respiratory system (trachea, gills) and/or via food. The latter route is specially important for lipophylic compounds. Insects are examples of species that are chemically well insulated from their environment, meaning that uptake via food is the dominant uptake route. This explains their seemingly insensitivity in acute toxicity tests, where test animals are not fed.

6. Exposure to chemical compounds

Now we have discussed stress by chemical compounds on the basis of their internal concentration, in the last subsection of the previous section, we finally need to consider the relationship between the internal concentration and the availability in the environment. In aquatic environments the latter is frequently specified as concentration in the water, where water is frequently quantified as volume, sometimes as gram. The specification of compounds in soils is frequently done as moles per gram of soil, but the link with availability for the organisms can be more complex and depends on the various uptake routes. Soils and sediments consist of smaller or larger silica-derived particles, frequently with an organic coating. Really dry soils have little active life and life activity is confined to the water around the silica particles. How much water can be in soils depends on the particle size in the first place. If there is a need to evaluate the toxicity of chemicals in sediments or soils, it is always wise to run exposures in water as a reference to separate soil chemistry from the actual toxicity of the compounds. Many insects, for example, seem rather insensitive to toxic stress in acute toxicity tests; they are typically not fed in these short-lasting tests to keep things chemically as simple as possible. Insects are, however, chemically rather well insulated from their environment, and most compound is in practice taken up via food. Their lack of sensitivity is thus in the first place a matter of exposure, not of intrinsic insensitivity. The conclusion is that enough attention should be given to how the compound actually reaches potential targets to access and interpret apparent sensitivity.

6.1. Availability

The previous section discussed chemical transformation in the organism, but we also need to discuss chemical transformation in the environment, since it affects uptake and so stress. This is captured by the label 'availability of compounds'.

The solubility of many compounds is very poor, meaning that the compound is present as dissolved as well as in solid form; chemicals can also leave the water and evaporate. The uptake routes of particles and dissolved forms can be very different. Nanoparticles recently became in large-scale use and the chemistry of these particles is known very partially only. Most of them have a coating with another compound and it is not even clear to what extend they can pass the gut wall. The study of effects of nanoparticles is under development (Li et al., 2011; Muller et al., 2014). In dissolved form, the compound can be present in molecular or in ionic form, and many compounds have the tendency to adsorb to (organic) particles and surfaces (including those of tanks that are used in experiments) and form ligands with organic molecules. The chemical environment in guts differs from that outside the animal, with consequences for the chemical behaviour.

Chemical compounds typically react with other compounds, can be thermally unstable, can transform by UV radiation (Rozema

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et al., 1999). Compounds can be transformed by (micro)organisms (Eichinger et al., 2006; Eichinger, 2008). Bio-degradation, or better phrased bio-transformation, can depend on the way the compound is present, dissolved or absorbed in/to particles (Brandt and Kooijman, 2000; Brandt, 2002), and on the availability of resources for micro-organisms, a phenomenon known as co-metabolism (Brandt et al., 2003). If you dose a chemical into an environment and directly try to measure its abundance, then your recovery fraction will be rarely more than 0.1. What chemical species you actually measure depends on the method that you use, including the preparation steps you apply to your samples. Water (fresh or salt) that is used in (aquatic) toxicity tests in the laboratory differs from that in the environment by having much less organic compounds with which the chemical can form ligands, much less particles at which it can absorb, much less microbes that can transform it, probably a different acidity, hardness and ionic strength. If organisms take up chemical compounds, we need to think about bio-availability of the various metabolites. If uptake is via food the problem of exposure can be really complex. This is one of the reasons for not feeding animals in acute toxicity tests, but this obviously affects realism of such test to predict effects in the environment.

6.2. Mixtures

My motivation to mention all this is that it is little naive to think about the toxicity of a single chemical compound, where actually a dynamically changing mixture of metabolites is involved (Jager et al., 2014; Baas et al., 2010a,b); almost all chemical compounds typically directly transform into a number of other compounds, e.g. ionic forms. In the previous section, I already discussed the issue that even when a single compound is taken up from the environment, it can become a mixture of metabolites in the body.

The methodology of studying effects of mixtures are presently well worked out (Jager et al., 2010; Baas et al., 2009; Baas, 2010). The general idea is that each compound in the mixture affects the target parameter with additive terms, and if compounds interact pairs-wise, this interaction contributes proportional to the product of their concentrations, just like usual in the Analysis of Variance. The product terms can be positive, or negative and all possible pairs are considered. The motivation is again by Taylor approximation of multi-variate functions, which applies well for small effect levels. This approach is only feasible for a mixture of a small number of compounds, since the number of required parameters rapidly increases with the number of compounds. Interactions are usually of minor importance and for complex mixtures they are ignored for convenience; the effect-parameters are subsequently assumed to be drawn for a simple (e.g. log-normal) distribution, so reducing the number of required parameters substantially.

The step from exposure under laboratory conditions to expected effects in the environment is quite big, both from a chemical and a biological point of view. Although mixture toxicity is generally thought to be a step up in complexity, compared to that of pure compounds, it is closer to the natural way we should think of toxicity of compounds.

6.3. Transport

Since compounds need to be in the organism to have potentially an effect, we need to think about transport, both within the environment and from the environment into the organism. Models for transport (of resources and chemical compounds) in the environment (sediments, water, air) substantially improved recently and now can be used to improve models for populations and ecosystems as well as their exposure to chemical compounds. I here discuss the transport from the (near) environment to the organism a bit further.

The ratio of the internal and external concentrations changes in time, but generally ultimately settles at what is known as the partition coefficient or bio-concentration factor. As always, we first need to think about its dimension: the ratio of the amounts of a compound in biomass and in the environment is gram of biomass per gram (or volume) of environment. This ratio is not dimensionless since it makes generally little sense to add mass of organism to mass of environment. The bio-concentration factor is frequently presented in the literature as if it is dimensionless, but this is incorrect.

The one-compartment model can considered to be the most simple transport model from which all other transport models are derived. It assumes that the uptake rate is proportional to the external concentration and the excretion (elimination) rate proportional to in the internal concentration. The two parameters, the uptake rate with dimension volume (or mass) of environment per time times volume (or mass) of biomass, and the elimination rate with dimension one over time, have very different dimensions. The elimination rate quantifies how fast the internal concentration satiates and can be extracted for e.g. survival or sub-lethal effect data. The uptake rate can only be obtained from chemical measurements. The bio-concentration factor is the ratio of the uptake and the elimination rate in this model.

Film-models represent a popular variation on one-compartment models to model transport in the environment, where transport between compartments is thought to be limited by films at both sides of the interface, where a concentration gradients build-up, contrary to the media outside the films, which are still assumed to be well-mixed. Here we have a process in time (transport between the compartments) and in space (in the films), which calls for partial differential equations to quantify transport. Although the concentration gradients are assumed to be in equilibrium within the (very thin) films, we still need the partial differential equation formulation to evaluate the jump in concentration over the interface. This jump changes over time towards an equilibrium value when a gradient in both films no longer exists and net-transport across the interface ceases. Despite the popularity of film models, I never found the formulation in terms of partial differential equations and we had to derive it ourselves (Kooijman et al., 2009a,b; Kooijman, 2010).

Multi-compartment models represent another variation of one-compartment models to model transport within biomass, specially in a pharmacological context. They are transport models, so they have the property that internal concentrations eventually reduce to zero in absence of compound in the environment. Yet, internal concentration profiles frequently show a decrease to some positive value during elimination after exposure. This pattern can only be captured with transformation of the compound, not with transport only. Many other variations of the one-compartment model have been formulated, e.g. to account for size and growth (change in size) and metabolism (Kooijman, 2010).

7. Meta-models

Meta-models are models for the values of parameters, where the same model is applied to a, potentially large, number of cases (e.g. species and/or compounds). I think of patterns in a (large) table of values for species (or compounds) versus parameters for some particular model. Meta-models can obviously only be composed for models that are sufficiently general to be applied to a range of cases, and the parameters must have clear physical interpretations to help understanding beyond description. So far, I discussed models that are based on mechanisms for underlying processes. These models have parameters, which tend to co-vary in different applications in ways that can sometimes be predicted without having seen any numerical result. This holds for both the

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standard DEB model (Kooijman, 1986) and the one compartment model (Kooijman et al., 2009a,b), which both can be seen as canonical forms: the simplest non-degenerated representation of much wider family of related models that have the same parameters and some more. As far as I know, this self-invented line of argument has only been applied to these two models and I am unsure if the line of reasoning can be applied to a much wider class of models. The purpose of this section is by showing the line of reasoning behind meta-modelling to expose the concept and to stimulate research in its wider applicability. A second motivation is to further structure the line of thinking from data to models to meta-models, so increasing our intellectual grasp on a complex reality.

The reasoning behind meta-models, in this particular context, has a number of layers, starting from plain physics, to more biological (evolutionary/ecological) and chemical arguments. The physical argument rests on the classification of parameters into intensive (i.e. not depending on absolute size) and extensive (i.e. depending on absolute size). The concept compound parameter is also essential in the reasoning, which is a simple function of parameters, and especially its physical dimensions and interpretation.

For the standard DEB model we first focus on maximum structural length $L_m = \frac{\kappa\{\dot{p}_{Am}\}}{[\dot{p}_M]}$, where specific somatic maintenance $[\dot{p}_M]$ and allocation fraction κ are intensive, with the implication that specific maximum assimilation $\{\dot{p}_{Am}\}$ must be extensive and proportional to maximum structural length L_m. The next step is to consider appropriate ratios of these and other parameters that are intensive and determine how the other parameters scale with L_m. Once we know how all parameters scale with L_m, we know how they co-vary. This situation is simple because L_m involves just a single extensive parameter: no freedom left.

The situation with the partition coefficient $P = i/\dot{k}_e$ is slightly more complex, because two extensive parameters are involved in this function of parameters: uptake rate i and elimination rate $\dot{k}_e$. We first make the reconstruction step where the compound is not moving from an infinitely large environment to a (single) compartment, but between two compartments. We then remove a degree of freedom by realising which of the two compartments represents the organism and which the environment is an arbitrary choice. Using this symmetry-argument we can derive that the uptake rate must be proportional to the square root of the partition coefficient and the elimination rate inversely proportional to the square root of the partition coefficient. Since film models are variations on one-compartment models, we can derive how their parameters co-vary.

Notice that in both cases, we arrived at co-variation rules by first expressing an asymptotic state, i.e. maximum structural length and partition coefficient, as a ratio of rate-parameters, and subsequently remove the link of parameter variations with this function by relating parameters directly to each other. Once we know how parameters co-vary, we can evaluate how functions of parameters co-vary with other functions of parameters. Examples of such functions for the standard DEB model are the maximum body weight and the respiration rate at that weight. For the one compartment model, we can think of the LC50 at some exposure time.

A large volume of literature exists on body size scaling relationships, where eco-physiological properties are log-log plotted against maximum body weight of species with the aim of getting the slope in a linear regression in the hope that it somehow relates to the Kleiber's value, 3/4, for respiration to explain how that property is controlled by respiration. There are several important differences with co-variation rules. First, body weight itself is not treated as an independent variable, but as a result of underlying processes. Second, the eco-physiological property is also expressed as a result of underlying processes. Third, the log-log plot does not need to be linear; it frequently resembles a linear function, however, just as a wide class of functions would do in a log–log plot. Fourth, no function is fitted; the curve can be drawn independently from the points on the basis of the parameter values of the generalized animal (Kooijman, 2010, Table 8.1), or on the basis of the mean parameter values of the species that are involved, after correction to $L_m = 1$ cm, from the add_my_pet collection. Deviations from the curve then reveal eco-evolutionary adaptations.

The same applies for quantitative structure-activity relationships, QSARs, where LC50's at some (fixed) exposure time are log-log plotted against the octanol–water partition coefficients, P_{ow} of chemical compounds. These P_{ow} values can nowadays better be computed from the structure of the molecule than measured; an important advantage. Biomass never contains octanol, however, but this compound has been selected as representing 'typical' biomass, with limited success. This habit is somewhat strange, because for small P_{ow}, the LC50 will be close to the ultimate value, the (external) no-effect concentration NEC, but for large P_{ow} the LC50 at this exposure time will still decrease dramatically with exposure time. The reason is that for small P_{ow}, the elimination rate is high, so the waiting time to steady state of the internal concentration is small. Although these empirical methods of data analysis can have some value in terms of interpolation, they will not contribute to a better understanding.

The applicability of co-variation rules in practice very much depends on the realism of the assumption that intensive parameters in different situations are the same. This is more likely among related species and among compounds with a related mode of action. Needless to say that ecological and evolutionary adaptations are not included in this reasoning, 'only' differences in size. These differences in size are, however, possibly the most dominant cause of variations in parameter values among species and with a range of 16 orders of magnitude difference among animal species, it is easy to see why. These expectations are of big help, however, to recognize adaptations in the comparison for species with a very different body size and in the effects of compounds with very different bio-concentration factors. The co-variation rules for model-parameters represent the most simple meta-model, defined as a model for parameter values of a model.

As said before, I see the physical argumentation for reasons of co-variation of parameter values as basic, but certainly not as exclusive. These rules can be combined with ideas on adaptation and selection for species, and with links to particular physical properties of compounds. Such meta-models can also concern models for how model-structure changed in evolution, see e.g. Kooijman and Troost (2007). These two meta-models for the parameters of the standard DEB model and the one-compartment model can cross-fertilize, as shown in Baas and Kooijman (2015), where the NEC of a collection of pesticides turns out to be inversely proportional to the specific maintenance $[\dot{p}_M]$.

8. Outlook

I see my field of Theoretical Biology as an interface between natural sciences, mathematics and philosophy of science with the primary task to optimize the way we arrive at conclusions that make sense in a wider context. An important first step in this is to make all assumptions explicit and to structure the use and testing of them. Over the many years I polished my ideas about the best way to organise the empirical cycle, which is not only meant to show empiricists the potential use of models, but also to show modellers how the function of models in science can be, if, and only if, models meet particular requirements.

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Most of my work has been in the ecological context of stressors on metabolism. In an early stage I already came to the conclusion that stress is a deviation of no-stress, which leads to the surprise that the ecotoxicological literature gives so little attention non-stressed situations. Test animals are typically seen as measurement-equipment to access particular properties of the compounds in standardized ways. Standardization is, however, not the solution to *understand* the dynamic nature of effects, while understanding is key to extrapolation and interpretation. I hope that this paper will motivate a change of such an attitude.

Acknowledgements

LabexMER and GDR-RECHAGLO funded the presentation of this paper. I want to thank Laure Pecquerie for organising this summerschool and, like the other curators of the add_my_pet collection, Starrlight Augustine, Dina Lika and Gonçalso Marques, for their inspiring and intense interaction.

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Please cite this article in press as: S.A.L.M. Kooijman, Models in stress research, Ecol. Complex. (2017), http://dx.doi.org/10.1016/j.ecocom.2017.07.006

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Please cite this article in press as: S.A.L.M. Kooijman, Models in stress research, Ecol. Complex. (2017), http://dx.doi.org/10.1016/j.ecocom.2017.07.006